*J. gen. Virol.* (1988), **69**, 777–786. *Printed in Great Britain*



Key words: *Hantaan virus/baculovirus/gene expression*

# Baculovirus Expression of the Small Genome Segment of Hantaan Virus and Potential Use of the Expressed Nucleocapsid Protein as a Diagnostic Antigen

By CONNIE S. SCHMALJOHN,[1]* KAZUYOSHI SUGIYAMA,[2] ALAN L. SCHMALJOHN[1] AND DAVID H. L. BISHOP[2]

[1] *Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011, U.S.A. and* [2] *NERC Institute of Virology, Mansfield Road, Oxford OX1 3SR, U.K.*

(*Accepted 18 December 1987*)



SUMMARY

*Autographa californica* nuclear polyhedrosis virus (AcNPV) was used as an expression vector for the nucleocapsid protein gene of Hantaan virus. Two different cDNA clones representing the small genome segment of Hantaan virus were inserted into the transfer vector pAcYM1, and recombinants were generated by replacement of a portion of the baculovirus polyhedrin gene with the foreign, Hantaan virus gene. Recombinants containing both the first and second ATG initiation codons of the Hantaan virus gene produced nucleocapsid protein, while those containing only the second codon did not. The expressed nucleocapsid protein was evaluated as a potential diagnostic antigen with a variety of hantavirus-immune sera. The high levels of expression obtained, specific serological reactivity with immune sera and the low level of biological containment required for production of this protein all suggest a significant advantage over authentic viral antigen for diagnosis of hantavirus infection.

## INTRODUCTION

The *Hantavirus* genus of the family Bunyaviridae includes the aetiological agents of Korean haemorrhagic fever (KHF), epidemic haemorrhagic fever (EHF), nephropathia epidemica (NE) and other clinically similar diseases, collectively termed haemorrhagic fever with renal syndrome (HFRS) (WHO, 1982). HFRS poses a significant disease threat in much of Asia, with annual reported hospitalized cases in excess of 50000 in mainland China (Song *et al.*, 1984), and 300 to 900 in Korea (Lee, 1982). Mortality rates vary, but have been reported to be as high as 5% to 18% in some areas of China (Jiang, 1983). Milder forms of HFRS generally occur in European and Scandinavian countries with few reported fatalities (Lahdevirta, 1982).

Antibodies to hantaviruses have been detected in rodents throughout most of the world, both in areas where HFRS is endemic and in regions where it has not been observed (LeDuc *et al.*, 1986*a*). Hantavirus infection of rodents is apparently non-pathogenic and persistent, and transmission to humans is believed to occur via aerosols of infectious virus from the animals' urine, faeces and saliva (Lee, 1982). Such viral persistence in naturally infected laboratory rats has resulted in numerous occurrences of HFRS among animal handlers and laboratory workers, and indicates the need for routine screening of rodents in colonies to ensure the absence of hantavirus infection (WHO, 1983).

Current hantavirus screening procedures rely either on the detection of antiviral antibodies by indirect immunofluorescence tests or by ELISA. Both methods require the production of diagnostic antigen under biological containment conditions and subsequent inactivation of infectivity. The hazardous nature of these viruses, their slow replication characteristics and their low yields in cell culture suggest the need for development of alternative means for antigen

production. The recently elucidated coding strategies of the medium (M) and small (S) genome segments of prototype Hantaan virus, and the availability of cDNA clones representing those genes, provide one such alternative: expression of viral proteins (Schmaljohn *et al.*, 1986, 1987*a*). In this report, we describe the expression of the S genome segment of Hantaan virus in an insect virus system and present evidence that the expressed nucleocapsid protein may be useful for the detection of antibodies to hantaviruses.

## METHODS

*Viruses, cells, and cDNA clones. Autographa californica* nuclear polyhedrosis virus (AcNPV) and recombinant viruses were propagated and assayed in *Spodoptera frugiperda* cells according to previously published methods (Brown & Faulkner, 1977). Hantaan virus, strain 76-118, was propagated in Vero E6 cells (Schmaljohn *et al.*, 1983). cDNA clones representing the S genome segment of Hantaan virus were as previously described (Schmaljohn *et al.*, 1986).

*Immune animal sera.* The preparation of hantavirus-immune sera in experimentally infected rats has been described (Schmaljohn *et al.*, 1985). Immune rabbit sera were generated by intramuscular inoculation of at least two New Zealand White rabbits with infectious Hantaan, Puumala, Prospect Hill or Seoul urban rat viruses. Rabbit sera were tested at weekly intervals for the presence of virus-neutralizing antibody, and high-titre sera were pooled as reference antisera for each of the four virus serogroups.

*Construction of the AcNPV recombinant transfer vectors and recombination with AcNPV.* Prior to ligation of Hantaan virus cDNA to the transfer vector, it was necessary to remove the homopolymeric tails originally added to the cDNA to facilitate cloning into the *Pst*I site of the plasmid pBR322 (Schmaljohn *et al.*, 1986). Because the cDNA representing the S segment of Hantaan has an internal *Pst*I site, the clone was excised from its parent plasmid by partial digestion with *Pst*I, subcloned into the *Pst*I site of the plasmid pUC-4K (Pharmacia), re-excised with *Bam*HI, and subjected to brief exonucleolytic digestion with Bal31 enzyme. The ends of the digested DNA were repaired with the large fragment of T4 DNA polymerase (Klenow) and ligated to the transfer vector pAcYM1 (Matsuura *et al.*, 1987), which had been made linear with *Bam*HI and repaired with Klenow enzyme. The junctions of resultant Hantaan virus–pAcYM1 transfer vectors were sequenced to confirm retention of the Hantaan virus initiation codon. Recombination by co-transfection of *S. frugiperda* cell cultures with transfer vectors and purified AcNPV viral DNA was as previously described (Overton *et al.*, 1987).

*Fractionation of infected cell lysates and polyacrylamide gel electrophoresis.* Two 25 $cm^2$ flasks of recombinant-infected or uninfected *S. frugiperda* cells were radiolabelled from 24 to 48 h post-infection with 200 μCi flask of [$^{35}$S]methionine in Grace's insect medium (Gibco) without foetal calf serum. Monolayers were washed once with Dulbecco's phosphate-buffered saline (PBS) (Gibco) prior to the addition of either 1 ml/flask of physiological saline or NET buffer (400 mM-NaCl, 50 mM-Tris–HCl pH 8·0, 1 mM-EDTA, 1% Triton X-100, 10 μg ml $\alpha_2$-macroglobulin, 10 μg/ml aprotinin), and cells were harvested by vigorous shaking. Cells suspended in saline were disrupted by 20 strokes in a tight-fitting, stainless steel Dounce homogenizer. The cell lysates were then subjected to crude fractionation by low speed centrifugation (500 ***g***) for 10 min followed by high speed centrifugation (13000 ***g***) for 15 min. The low speed and high speed pellets were resuspended in 1 ml of saline and 50 μl aliquots of each of the pelleted fractions and the remaining supernatants were analysed by polyacrylamide gel electrophoresis as previously described (Schmaljohn *et al.*, 1983).

*Antigen treatment for ELISA.* Recombinant-infected *S. frugiperda* cells were harvested 4 days post-infection when c.p.e. first became apparent. Approximately 2 × $10^7$ cells were pelleted by centrifugation at 3000 ***g*** at 4 °C, resuspended in 1 ml of serum-free Grace's insect medium and sonicated for 1 min with a microtip (Fisher Scientific, Fairlawn, N.J., U.S.A.) at 35% maximum output while maintaining samples on ice. Disrupted cells were clarified by centrifugation at approximately 1000 ***g***, and 100 μl aliquots were diluted with an equal volume of each of the following: double distilled (dd) water; 2% SDS in water, 1·0 M-NaCl; buffer A (0·2 M-$Na_2PO_4$, 0·15 M-NaCl pH 9·0); buffer B (0·2 M-$Na_2PO_4$, 0·15 M-NaCl pH 4·1); or 2% Triton X-100 in water. Samples were incubated for 15 min at room temperature and centrifuged in an Eppendorf microcentrifuge for 2 min. Twofold dilutions of each antigen preparation were made in PBS, and 100 μl aliquots of each were dried onto wells of standard, flat-bottom ELISA microtitre plates.

*ELISA.* Sera from rabbits, rats and humans were tested in duplicate for the ability to bind nucleocapsid antigen in conventional ELISAs, which were performed as previously described (Stec *et al.*, 1986) with the following minor modifications: PBS containing 10% (w/v) non-fat dry milk was substituted for bovine serum albumin for pretreatment of antigen-containing wells (blocking), and 100 μl, rather than 50 μl, of each antigen or antibody was placed in each microtitre well. Peroxidase-labelled anti-rabbit IgG, IgA and IgM (Cappel Laboratories), anti-rat IgG (Kirkegaard & Perry, Gaithersburg, Md., U.S.A.), or anti-human IgG (Accurate Scientific Corp., Westbury, N.Y., U.S.A.) were diluted 1:16000, 1:1000 and 1:16000, respectively, and these enzyme-linked secondary antibodies were detected with ABTS substrate (Kirkegaard & Perry).

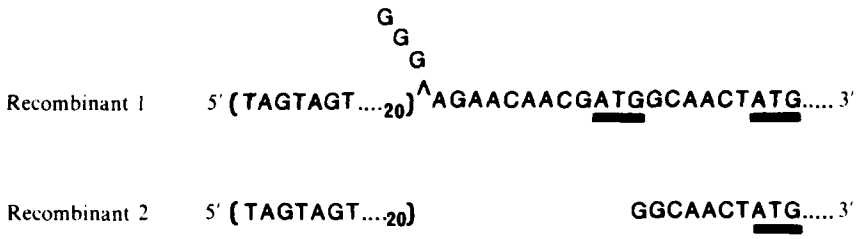


Fig. 1. Hantaan virus sequences at the 5′ ends of the two pAcYM1–Hantaan virus S transfer vectors. Bracketed nucleotides represent the first 27 nucleotides of the 5′ end of Hantaan virus cDNA corresponding to the viral complementary-sense RNA. These nucleotides were not present in the Hantaan virus cDNA clones used for recombination. Three of the 13 G residues of the homopolymeric tail remained in recombinant 1 after Bal31 digestion. All of the G residues as well as 11 additional bases were removed from recombinant 2. The two potential ATG initiation codons are underlined.

*Southern blot analysis.* AcNPV and recombinant viral DNAs were purified as previously described (Overton *et al.*, 1987). After digestion of DNA with various restriction enzymes and resolution of fragments by agarose gel electrophoresis, the DNA was electroblotted to Gene Screen Plus hybridization membranes (DuPont). Radiolabelled probes were prepared from the internal *Bam*HI fragment of Hantaan virus cDNA (representing nucleotides 138 to 1242) by using random oligonucleotide primer synthesis (Pharmacia). Membranes were prehybridized and hybridized according to the manufacturer's directions.

## RESULTS

### *Construction of Hantaan virus–baculovirus recombinants*

Previous studies demonstrated that the level of expression of certain foreign viral genes by baculovirus recombinants is directly related to the 5′ upstream sequences of the polyhedrin gene (Matsuura *et al.*, 1987). Among those examined, the highest expression levels were obtained with the transfer vector pAcYM1, which contains upstream coding sequences of the AcNPV polyhedrin gene, including the A of the initiating ATG codon. Consequently, pAcYM1 was chosen for Hantaan virus–AcNPV recombination.

Two in-frame ATG initiation codons have been reported to occur at nucleotide positions 37 and 46 of the viral complementary sense RNA of the S segment of Hantaan virus (Schmaljohn *et al.*, 1986). Both codons have equally favourable flanking sequences for initiation of protein synthesis, and it is not known which of the two is actually used for synthesis of the Hantaan virus nucleocapsid protein. Consequently, two pAcYM1–Hantaan virus transfer vectors were constructed, one of which included 12 bases upstream from the first ATG initiation codon, and a second one which contained seven bases upstream from the second ATG, but did not include the first initiation codon (Fig. 1). After tranfection of *S. frugiperda* cells with mixtures of AcNPV DNA and transfer vector DNA, progeny recombinant viruses were identified by production of polyhedrin-negative plaques and were plaque-purified three times on monolayers of *S. frugiperda* cells.

To verify the presence of the Hantaan virus S genome segment in the recombinants, purified AcNPV or recombinant viral DNAs were digested with the restriction enzymes *Pvu*I, *Bam*HI, or a combination of *Eco*RV and *Cla*I, and Southern blots were prepared and hybridized with radiolabelled Hantaan virus S cDNA probes. *Pvu*I has a unique restriction site in pAcYM1 but not in Hantaan virus S cDNA. Digestion with this enzyme yielded a large DNA fragment which was identifiable in both recombinant DNAs, but not in AcNPV DNA, after hybridization with the Hantaan virus probe. *Bam*HI digestion of both recombinants generated a DNA fragment identifiable by hybridization of approximately 1·1 kb, which corresponded to Hantaan virus cDNA between two internal *Bam*HI sites located at nucleotides 138 and 1242. Similarly, the double digestions with *Eco*RV and *Cla*I (sites for which are not present in Hantaan virus cDNA) generated a fragment of approximately 2·4 kb, which corresponded to the entire 1·7 kb Hantaan virus S segment plus about 700 base pairs of AcNPV DNA (Fig. 2).

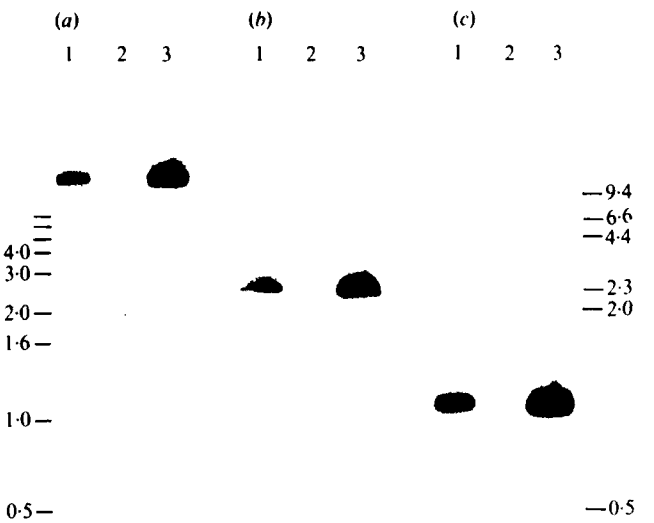


Fig. 2. Southern blots of purified recombinant viral DNA. Viral DNA from recombinant 1 (lanes 1), AcNPV (lanes 2), or recombinant 2 (lanes 3) was digested with restriction enzymes (*a*) *Pru*I, (*b*) *Cla*I and *Eco*RV and (*c*) *Bam*HI and hybridized with $^{32}$P-labelled Hantaan virus cDNA probes as described in the text. Fragment sizes are given in kb.

### *Expression of Hantaan virus nucleocapsid protein*

To determine whether the recombinants were able to express the foreign Hantaan virus gene, recombinant-infected *S. frugiperda* cells were examined by indirect fluorescent antibody staining with polyclonal and monoclonal antibodies to Hantaan virus. Immunofluorescence was observed in cells infected with recombinant 1 (i.e. those which contained Hantaan virus sequences, including the first ATG codon) (Fig. 3), but not in cells infected with recombinant 2 (constructs initiating after the first ATG) or in cells infected with wild-type AcNPV (not shown). Similarly, immune precipitation revealed a protein indistinguishable from authentic Hantaan virus nucleocapsid protein in radiolabelled lysates of cells infected with recombinant 1 (Fig. 4), but no polypeptides were precipitated from recombinant 2-infected cell lysates (not shown).

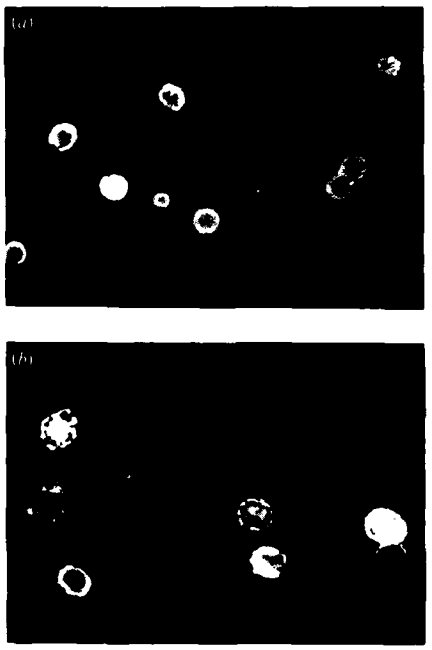

Fig. 3

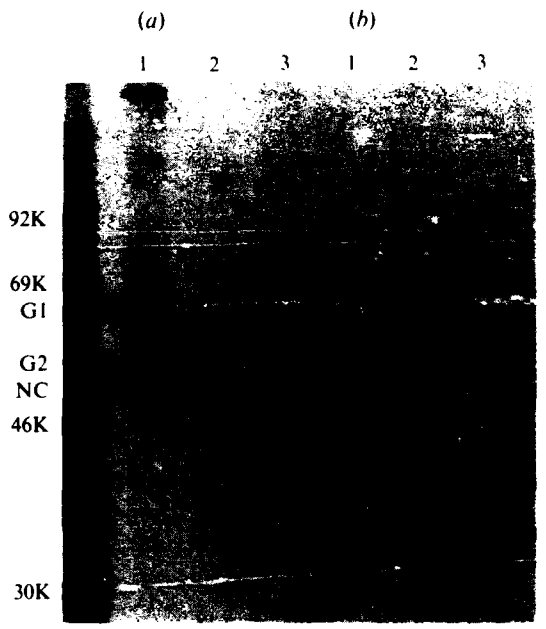


Fig. 4

Fig. 3. Indirect immunofluorescence of recombinant-infected *S. frugiperda* cells reacted with polyclonal (*a*) or monoclonal (*b*) antibodies.

Fig. 4. Comparison of expressed Hantaan virus nucleocapsid protein to authentic nucleocapsid protein by polyacrylamide gel electrophoresis. Radiolabelled polypeptides were immune precipitated with polyclonal hyperimmune mouse ascitic fluid (lanes 1), a pool of five anti-nucleocapsid monoclonal antibodies (lanes 2), or with normal mouse serum (lanes 3). Arrows indicate authentic Hantaan virus envelope proteins (G1 and G2) or nucleocapsid protein (NC).

Because the foreign gene was under the control of the promoter normally used for production of the baculovirus polyhedrin protein, it was of interest to compare the relative amounts and distribution of expressed protein in recombinant-infected cells with those of the polyhedrin protein in AcNPV-infected cells. Crude fractionation of radiolabelled, infected cell lysates after non-ionic detergent disruption or Dounce homogenization was performed by centrifugation; the high and low speed pellets and supernatant fractions were examined by polyacrylamide gel electrophoresis. Although both the polyhedrin protein and the expressed nucleocapsid protein could be detected in soluble and pelleted cell fractions, the majority of each was found in association with cell pellets (Fig. 5*a*). Densitometric tracings of the autoradiograph lanes representing pelleted fractions revealed that approximatley 50% of the radiolabelled polypeptides in the recombinant-infected cells were the expressed protein and about 65% of the radiolabelled polypeptides in the AcNPV-infected cells were the polyhedrin protein (Fig. 5*a*). In contrast, authentic Hantaan virus proteins could not be detected above the background of radiolabelled, host cell proteins (Fig. 5*b*). Similar results were obtained by staining polyacrylamide gels with Coomassie Brilliant Blue; i.e. the expressed nucleocapsid protein was the major polypeptide observed, while authentic nucleocapsid protein could not be discerned above host cell proteins (not shown).

### *Antigenicity of the expressed nucleocapsid protein*

To investigate the potential utility of the expressed nucleocapsid protein as a diagnostic antigen, lysates were prepared from recombinant-infected *S. frugiperda* cells and standard

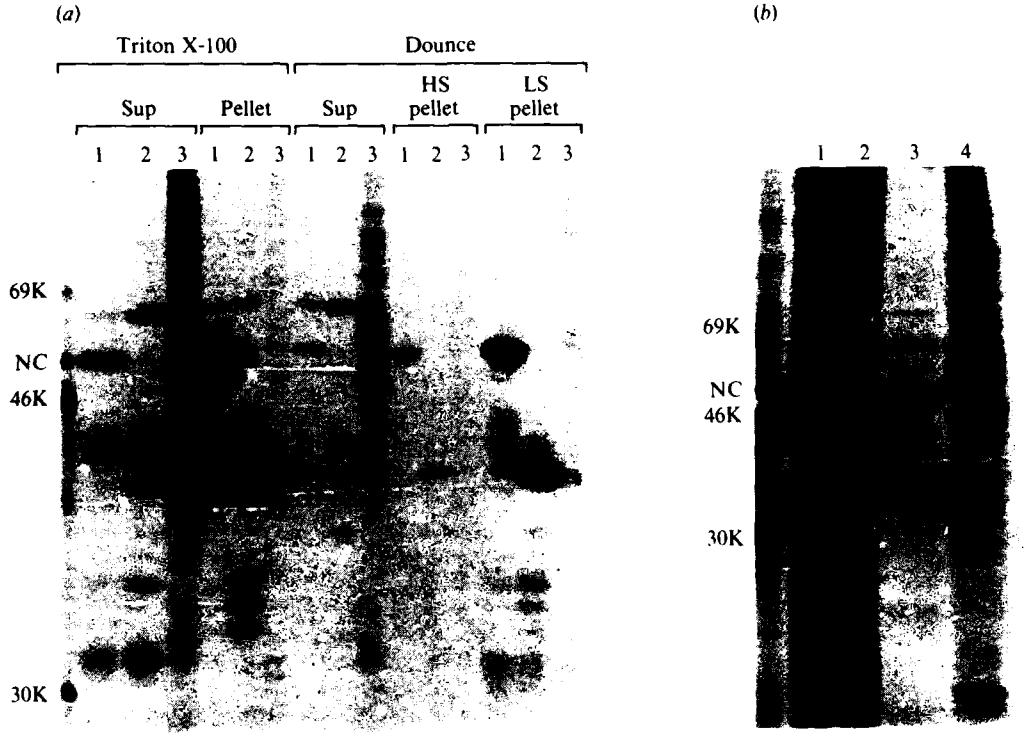


Fig. 5. Comparison of relative amounts of radiolabelled expressed Hantaan virus nucleocapsid protein to those of AcNPV polyhedrin protein (*a*) and to authentic Hantaan virus nucleocapsid protein (*b*). (*a*) Recombinant-infected (lanes 1), AcNPV-infected (lanes 2) or uninfected (lanes 3) *S. frugiperda* cells were disrupted by Triton X-100 treatment or Dounce homogenization and fractionated by centrifugation as described in the text. Sup, supernatant; HS, high speed; LS, low speed. (*b*) Total cytoplasmic extracts of Hantaan virus-infected Vero E6 cells (lane 1), uninfected Vero E6 cells (lane 2), recombinant-infected *S. frugiperda* cells (lane 3), or uninfected *S. frugiperda* cells (lane 4) were compared by polyacrylamide gel electrophoresis of radiolabelled polypeptides.

ELISA reactions were performed with anti-Hantaan virus hyperimmune mouse ascitic fluid. The recombinant-infected lysates reacted to high titre with anti-Hantaan virus but not normal ascitic fluid, while control cell lysates from uninfected or AcNPV-infected cultures were unreactive (Fig. 6).

Unlike authentic Hantaan virus nucleocapsid protein, which is a major viral structural component and thus is found extracellularly in virion particles, the majority of the baculovirus-expressed protein was found to be cell-associated (data not shown). Various means of disruption of the cells, therefore, might have a significant effect on the relative amount and/or quality of available ELISA-reactive antigen. To compare the effects of various antigen treatments on ELISA titres, infected cells were sonicated then incubated with several detergents or buffers prior to ELISA. Among conditions tested, treatment with 1% SDS or 0·5 M-NaCl released the highest titre ELISA antigen response as measured with both anti-Hantaan virus or anti-nucleocapsid-specific polyclonal sera (Table 1).

*Diagnostic potential of the expressed protein for hantaviruses other than Hantaan virus*

More than one hantavirus capable of causing HFRS may circulate simultaneously in the same geographical area (Lee, 1982). To determine whether the expressed protein could serve as a diagnostic antigen for viruses serologically related to Hantaan virus, antisera to a representative virus of each of the four antigenic groups within the hantavirus genus were produced in rabbits or rats, and examined by ELISA. Both species of animals developed high neutralizing antibody

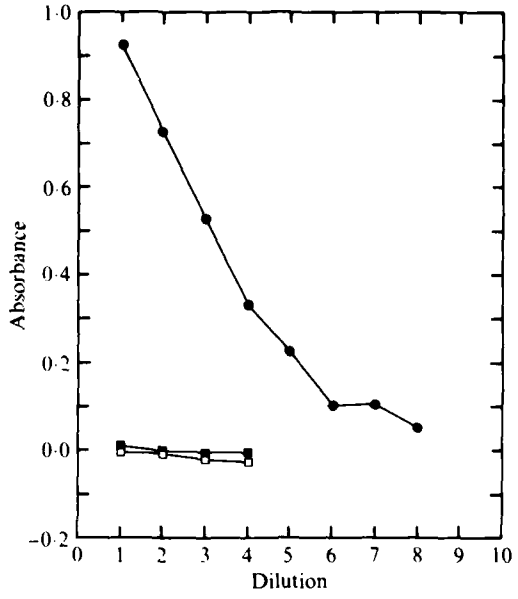


Fig. 6. Antigenicity of the expressed nucleocapsid protein. Twofold dilutions (1:50 to 1:6400) of recombinant-infected (●), uninfected (□) or AcNPV-infected (■) cell lysates were reacted with a constant amount of anti-Hantaan hyperimmune mouse ascitic fluid and analysed by ELISA.

Table 1. *Effects of antigen treatment on ELISA reactivity of expressed nucleocapsid protein*

| | Immune sera | | |
|---|---|---|---|
| Treatment | Hantaan virus | Nucleocapsid | Normal |
| $ddH_2O$ | 800* | 400 | <50 |
| 1% SDS | 12800 | 6400 | 50 |
| 0·5 M-NaCl | 6400 | 3200 | <50 |
| pH 9·0 | 3200 | 3200 | <50 |
| pH 4·1 | 800 | 400 | <50 |
| 1% Triton X-100 | 3200 | 800 | <50 |

* ELISA titres are defined as the reciprocal of antigen dilutions that generated absorbance readings >0·5.

Table 2. *Detection of expressed nucleocapsid antigen by hantavirus-immune rat and rabbit sera*

| | Immune sera | | | | |
|---|---|---|---|---|---|
| Animal | Hantaan virus | Seoul virus | Puumala virus | Prospect Hill virus | Normal |
| Rat | 3200* | 6400 | <50 | <50 | <50 |
| Rabbit | 1600* | 3200 | 50 | 400 | <50 |

* ELISA titres are displayed as the reciprocal of the dilution of serum giving absorbance readings >0·1 in excess of those obtained with AcNPV-infected cells at the same dilution.

responses to homologous virus, suggesting that infection had occurred (Schmaljohn *et al.*, 1985 and data not shown). Reactivities of the immune rat and rabbit sera with expressed nucleocapsid protein (Table 2) were similar to that previously observed with authentic Hantaan virus protein; that is, high titre responses were observed with immune sera to Hantaan and Seoul urban rat viruses but either negative or greatly reduced reactivities were found with Puumala virus and Prospect Hill virus immune sera (Schmaljohn *et al.*, 1985).

Table 3. *ELISA reactivities of HFRS patient sera and control human sera with expressed nucleocapsid antigen*

| Sample no. | Disease | Origin | ELISA titre* |
|---|---|---|---|
| 1 | KHF | Korea | >6400 |
| 2 | KHF | Korea | 3200 |
| 3 | KHF | Greece | 3200 |
| 4 | Inapparent† (laboratory infection) | U.S.A. | 800 |
| 5 | NE | Sweden | 800 |
| 6 | NE (laboratory infection) | Finland | 200 |
| 7 | Control | U.S.A. | <50 |

* ELISA titres are expressed as the reciprocal of the dilution of antisera resulting in absorbance readings >0·1 in excess of those obtained with control AcNPV-infected cultures at the same dilutions.

† Infection with Hantaan virus occurred in 1984; serum sample analysed was collected in 1987.

*Reactivities of HFRS patient sera with the expressed nucleocapsid protein*

A panel of human sera was screened by ELISA with the expressed antigen to test its potential suitability for use in disease diagnosis or serological surveys (Table 3). Among the seven sera examined, the highest titre response was obtained with an acute phase specimen from an American soldier stationed in Okinawa, Japan, who had contracted severe KHF in 1986, presumably whilst on manoeuvres in Korea (sample 1). High titre responses were also observed with sera from two other KHF patients, one of whom was infected while stationed in Korea in 1981 (sample 2) and the other, who was believed to have been infected in Greece and suffered a severe course of disease (sample 3) (Antoniades *et al.*, 1984; LeDuc *et al.*, 1986*b*). A positive ELISA response was also obtained with serum collected 3 years after an initial inapparent laboratory infection with Hantaan virus (sample 4). Much lower responses were observed with sera from two NE patients, one of whom acquired the disease in Sweden (sample 5) and the other who developed HFRS in Finland after performing laboratory experiments with Puumala virus-infected rodents (sample 6) (Brummer-Korvenkontio *et al.*, 1980).

## DISCUSSION

Hantaan virus is the prototype of the newly established *Hantavirus* genus of the family Bunyaviridae. Like other viruses in the family, Hantaan virus has a three-segmented, single-stranded RNA genome which encodes two envelope glycoproteins and a nucleocapsid protein in the viral-complementary sense RNA of the M and S genome segments, respectively (Schmaljohn *et al.*, 1983, 1986, 1987*a*). Four serological groups within the genus are currently recognized, the representatives of which are Hantaan, Seoul urban rat, Puumala and Prospect Hill viruses (Schmaljohn *et al.*, 1985). In addition to serological cross-reactivity, partial nucleotide sequence conservation of both the M and S genome segments of viruses in each of these four serogroups was recently established using nucleic acid probes (Schmaljohn *et al.*, 1987*b*). Expressed proteins from either of the two segments, therefore, might serve as a diagnostic antigen useful for detection of antibodies to a variety of hantaviruses. Expression of the S genome segment, however, presents several advantages over M segment expression, in that the nucleocapsid protein is the sole gene product, is not glycosylated and appears to require no processing (Schmaljohn *et al.*, 1986). In contrast, both envelope proteins arise from a single, continuous, open reading frame in the M genome segment, and must be co-translationally or post-translationally processed to yield the mature glycoproteins (Schmaljohn *et al.*, 1987*a*).

Although a variety of prokaryotic and eukaryotic vectors are now available, the baculovirus system and pAcYM1 transfer vector were chosen for Hantaan virus S genome expression because of the reportedly high levels of expressed product attainable, and the relative safety and ease of antigen production (Matsuura *et al.*, 1987). Of the two Hantaan virus AcNPV recombinants examined, only the one which retained both of the in-frame ATG initiation

codons was found to express the Hantaan virus nucleocapsid protein. It is not clear why the second recombinant did not express the protein, and the absence of the 14 nucleotides present in the first recombinant may be unrelated to the observed absence of expression. Previous attempts to identify the amino terminus of authentic Hantaan virus nucleocapsid protein by terminal sequence analysis have failed (unpublished data) and, therefore, it is not known whether the three amino acids encoded prior to the second ATG are part of the nucleocapsid protein. If so, it is possible that translation from the first ATG may be required for synthesis of a stable protein.

Evaluation of the expressed nucleocapsid protein for antigenicity and cross-reactivity among various representative hantaviruses revealed strong reactivity with antibodies to Hantaan and Seoul urban rat viruses and appeared quite useful for detection of antibodies in the sera of KHF patients. Less reactivity was evident with antibodies to Puumala and Prospect Hill viruses and with NE patients' sera. These results were not unexpected and reflect cross-reactivities observed with authentic Hantaan viral antigen, which contains not only nucleocapsid protein, but the envelope glycoproteins as well (Schmaljohn *et al.*, 1985).

The data presented here demonstrate the feasibility of producing Hantaan virus diagnostic antigen by gene expression. Further studies on the antigenic and functional properties of the expressed nucleocapsid protein and a more detailed comparison to those of authentic Hantaan virus nucleocapsid protein are in progress. This work should allow the identification of antigenic regions which are conserved among, or unique to, individual hantaviruses and may lead to the development of a variety of expressed proteins useful for group-reactive or virus-specific diagnostic antigens.

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(*Received 9 November 1987*)